

Fig. 8. View of the integuments at the micropyle as seen in the longitudinal median section of the corn. $\times 325$.

Fig. 9. Transverse section of coats, etc., in the middle region of the ovule of the corn. The line of compacting in the ovary wall has formed, the innermost cells are freed, the inner integument is compressed, the epidermis of the nucellus remains. $\times 325$.

PLATE XXVI.—Fig. 10. Transverse section of the coats of the corn grain at maturity after treatment with cold potassic hydrate. $\times 400$.

Fig. 11. Transverse section through the coats of the mature oat grain showing the union of the remaining cells of the ovary wall with the inner integument. The layer to the left of *i* is the inner integument; to the right, the epidermis of the nucellus. $\times 465$.

Fig. 12. Transverse section of the inner coverings of the mature wheat grain, showing union of the chlorophyll cells of the ovary wall with the inner integument after treatment with KOH. $\times 735$.

Fig. 13. Transverse section of the coverings of the mature wheat grain. $\times 465$.

Contribution to the biology of the organism causing leguminous tubercles.

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WITH PLATES XII–XV.

[Continued from p. 166.]

Historical resume.

Early period of investigations.

The tubercles were at first supposed to be normal parts of the plants on which they were found. According to Vuillemin⁶, De Léchamp⁷ named the genus *Ornithopus* from the character of the tubercles and De Candolle⁸ uses the form of the tubercles as one of the characters of a variety of *Ornithopus perpusillus*.

Bivona⁹ took them to be fungi of the genus *Sclerotium* and distinguished two types, the simple ones as *S. lotorum* and the lobed ones as *S. medicaginum*. Persoon's *Sclerotium rhizogonum*¹⁰ was also founded upon certain of these tubercles. Fries¹¹ rejects Persoon's *S. rhizogonum* referring the forms on

⁶Les tubercles radicaux des Légumineuses. Ann. d. Sci. Agronom. franc. et étrang. 1888. p. 96.

⁷Histoire générale des plantes. 1615.

⁸Prodromus Syst. Nat. Reg. Veg. II (1825). 312.

⁹Pugill. plant. rar., Siculæ, IV. 26.

¹⁰Traité sur les champignons comestibles, Paris. 1818.

¹¹Systema Mycologicæ, II. 250.

which it was based to growths from the roots of *Vicia*, etc. Fries however retains Bivona's two species *S. lotorum* and *S. medicaginum* and it was reserved for Tulasne¹² to cast them out of the category of fungi. To A. P. DeCandolle¹³ they resembled small fungi like *Sclerotium* but he regarded them as diseased outgrowths and Wigand¹⁴ recently has looked upon them as pathological forms.

Jessen¹⁵ doubts their parasitic origin, and Clos¹⁶ believed them normal structures with a physiological role and called them lenticels.

Others saw in them the products of animal parasitism, Malpighi¹⁷ giving to insects the credit of their origin while Cornu¹⁸ preferred to charge them to the account of *Anguillula*. Later he withdrew the charge and treated them as root branches.¹⁹

Those who have argued that the tubercles were normal organs of the Leguminosæ hold very diverse views as to their function in the plant economy. Gasparini²⁰ describes them as swollen lateral roots and with Kolaczek²¹ thought they were organs for absorbing food. DeVries²² and Wittmack²³ interpreted them as lateral roots with dwarfed growth, while Treviranus²⁴ judged they were imperfect buds which in case

¹²Fungi Hypogaei. 1851.

¹³Mémoire sur les Légumineuses. 1825.

¹⁴Bakterien innerhalb der geschlossenen gewebes der knollenartigen Anschwellungen der Papilionaceen Wurzel. Bot. heft. Forsch. aus dem Bot. Gart. Z. Marburg, 1887, p. 88, cited by Pichi, Atti della Società Toscani di scienze naturali, 1888.

¹⁵Sitzungsbericht d. Bot. Verein d. Prov. Brandenburg, 1878. p. 54.

¹⁶Ebauche de la rhizotaxie, 1848; Du collet dans les plantes et de la nature de quelques tubercles. Ann. d. Sci. Nat. Bot. III. XIII. 1849.

¹⁷Anatome Plantarum pars sec. de Gallis. Opera I. 1687, cited by Vuillemin, l. c. See also Sorauer, Pflanzenkrankheiten. I. 744; Frank, Krankheiten der Pflanzen.

¹⁸Commission du Phylloxéra. 1876.

¹⁹Etudes sur le Phylloxera vastatrix. Memoires de l'Académie des sciences. XVI. 1878.

²⁰Osservazioni sulla struttura dei spongjolari di alcune piante leguminose, Lett. all. Ac. di Napoli, 1851; cited by Tschirch, Ber. d. deutsch. bot. Gesells. (1887). 58.

²¹Lehrbuch der Botanik, 1856. p. 374.

²²Wachstumsgeschichte des roten Klees. Landw. Jahrb. von Theil 6, I, 1877; Verhandlung d. Bot. Vereins d. Prov. Brandenburg. 1878. p. 54-55.

²³Verhandlung d. Bot. Vereins d. Prov. Brandenburg. 1874. p. 54.

²⁴Ueber die Neigung der Hülsengewächse zu unterirdischer Knollenbildung. Bot. Zeit. 1853, p. 393. See Vuillemin.

the plants did not fruit could themselves develop into perfect plants. According to Nobbe,²⁵ Lachman,²⁶ Mattiolo e Buscalioni²⁷ they were places for the storage of reserve food materials.

These notions were probably empirical and derived more from superficial examination than from any serious attempt at an anatomical study of their structure and contents.

Middle period of investigations.

The period of serious investigation into their structure and etiology begins with Woronin's²⁸ contribution in 1866. He described the tubercles as being composed mainly of a parenchymatous tissue which is separated into an inner central mass and an outer layer, the two portions being separated by a layer of fibro-vascular tissue which arises from corresponding tissue in the central cylinder of the root. The inner parenchyma he described as possessing turbid contents while the outer was clear. The growing point or meristem of the tubercle lies in the distal portion of the inner parenchyma, the proximal portion being occupied by bacteria-like bodies or vibrios which filled the plasmic substance. These he considered to be the cause of the tubercles.

The next important contribution was by Eriksson²⁹ who distinguished what he supposed were two organisms in the tubercles. One he verified as the vibrio-like bodies discovered by Woronin which occupy the proximal portion of the central parenchymatous tissue. In the distal portion of the central parenchymatous tissue he discovered what he took to be fungus strands lying in a radial position, branching into finer threads as they approached the center. He described

²⁵Vegetationsversuche in Boden mit lokalisirten Nährstoffen. Landwirthschaftliche Versuchstationen, 1868, p. 98.

²⁶Ueber Knollchen der Leguminosen. Landw. Mittheil. Zeitschr. d. k. Lehranstalt und Versuchstationen, Poppelsdorf, 1856, p. 37. Cited by Tschirch, l. c.

²⁷Si Contengono bacteri nei tubercoli radicati delle Leguminose? Malpighia, 1, 27, 464, cited by Pichi, l. c.

²⁸Ueber die bei der Schwarzerle und der gewöhnlichen Garten-Lupine auftretenden Wurzelanschwellungen, Mem. d. l' Acad. Imp. d. Sciences, VII. 1 (1866) 2.

²⁹Studier öfver Leguminosernas rotknölar, Lund, 1874; Bot. Zeitung, 1874, p. 381. Cited by Vuillemin, l. c. and others

also the characteristic enlargements of these structures where they pass through the cell walls. He believed these fungus hyphæ to be the real cause of the tubercles.

DeVries³⁰ observed the hyphæ described by Eriksson but did not believe they stood in causal relation to the tubercles.

Schindler³¹ also detected the hyphæ but claimed they enter after the tubercles begin to degenerate, while Cornu³² and Mattei³³ declare there exists no mycelium in the tissues.

Kny³⁴ recognizes the presence of strands but denies the presence of a membrane. He believed they were strands of a plasmodium similar to Woronin's *Plasmodiophora brassicæ*³⁵ and about this time Woronin³⁶ retreats from his former position that the tubercles were caused by bacteria or vibrio-like bodies, and believes them to be due to a plasmodium, being influenced probably by his studies upon the club-foot of cabbage.³⁷ Prillieux³⁸ largely corroborated the studies of Eriksson, especially in regard to the structure of the tubercles and their parasitic origin, but believed them to be caused by a plasmodium, related to Woronin's *Plasmodiophora brassicæ*, which excretes a viscid substance.³⁹

In 1879⁴⁰ appeared B. Frank's first important paper on the subject. He found both the hyphæ and the bacteria-like bodies. He believed the latter sprouted off from the former. In an earlier publication⁴¹ he thought the fungus to be closely allied

³⁰Wachstumsgeschichte des roten Klees. Landw. Jahrb. 1877.

³¹Ueber die biologische Bedeutung der Wurzelknöllchen bei den Papilionaceen. Jour. f. Landw. xxxiii, (1885), 325-336. Ueber die Bedeutung der sog. Wurzelknöllchen bei den Papilionaceen. Oesterreich. landw. Wochenblatt (1885), n. 34, cited by Tschirch, l. c. Zur Kenntniss der Wurzelknöllchen der Papilionaceen. Bot. Centralb. xviii (1884), 86.

³²Mem. d. l' Acad. d. Sciences, xxvi (1878).

³³ Ancora sull. origine della Vicia faba. Bologna, 1887, cited by Vuillemin, l. c.

³⁴Verhandlung. d. Bot. Vereins der Prov. Brandenburg, 1877. Ueber die Wurzelanschwellungen der Leguminosen und ihre Erzeugung durch Einfluss von Parasiten. Ibid. 1878.

³⁵Pringsheim's Jahrb. f. wiss. Bot. xi, 548.

³⁶Ibid., p. 371.

³⁷Ibid., p. 548.

³⁸Sur la nature et sur la cause de la formation des tubercles qui naissent sur les racines des Légumineuses. Bull. Soc. Bot. d. France, 1879, p. 98.

³⁹Comptes Rendus d. sc. Paris, cxi, 926.

⁴⁰Ueber die Parasiten in den Wurzelanschwellungen der Papilionaceen. Bot Zeitung. xxxvii (1879), n. 24, 25.

⁴¹Lennis: Synopsis: Kryptogamen, p. 1,944.

17-Vol. XVIII. - No.

to the genus *Protomyces* of De Bary,⁴² but now he transfers it to the genus *Schinzia* founded by Nægeli⁴³ on a fungus which causes galls on the roots of *Cyperus*, and calls it *Schinzia leguminosarum*, thus considering it to be closely related to the fungus in galls on the roots of *Alnus* first described by Woronin.⁴⁴ It is quite important in view of Frank's change of opinion at a subsequent date to note that an examination of the illustrations and descriptions which accompany this paper leaves no doubt that at that time he considered the bacteria-like bodies to be budded off from the hyphæ though he did not actually see the budding take place. This paper of Frank's brought out a rejoinder from Kny⁴⁵ who states that the bacteria-like bodies are the spores of the plasmodium.

The general effect of these investigations was to give a feeling of confidence in the view that the etiology of the tubercles was primarily due to the irritating or stimulating influence of some organism in the soil, though there was great diversity of opinion as to the character of the organism.

This feeling of confidence, however was soon shaken by the appearance in 1885 of a paper by Brunchorst.⁴⁶ Brunchorst claims that the tubercles are not caused by any organism. He affirms the older views that they are normal structures. To the vibrio-like bodies he ascribes an entirely new role. He believes them to be differentiated portions of the plasmic protein contents of the cells. While they possess the form of bacteria they are inert and serve as receptacles for the storage of proteid substance by the plant since they were found to be exceedingly rich in protein matter and that later their contents are absorbed by the plant. Because of their morphological resemblance to bacteria Brunchorst termed them bacteroids. He observed the fungus hyphæ but did not consider them to stand in causal relation to the tubercles since he found none

⁴²Beiträge zur Morph. u. Phys. d. Pilze, 1.

⁴³Sur des Champignons vivant dans l'intérieur des cellules végétales, *Linnæa*, 1842, p. 278. Translated in *Ann. Sci. Nat. Bot.* II, XIX (1843), 86.

⁴⁴Observations sur certaines excroissances que présentent les racines d' *Alnus* et d. *lupin* des jardin. *Ann. d. Sci. Nat. Bot.* V. VII, 73. See also footnote 14.

⁴⁵Zu dem Aufsatz des Herrn Prof. B. Frank über Parasiten in den Wurzelanschwellungen der Papilionaceen. *Bot. Zeit.* 1879. p. 537.

⁴⁶Ueber die Knöllchen an den Leguminosenwurzeln. *Ber. d. Deutsch. Bot. Gesells.* III (1885). 241-257.

in the tubercles of lupines. He as well as Eriksson⁴⁷ showed that two types of structure existed, one represented by the tubercles on lupine, and the other by those on peas, etc.

Schindler,⁴⁸ Tschirch,⁴⁹ Benecke,⁵⁰ Van Tieghem and Duiot,⁵¹ and Sorauer⁵² supported Brunchorst's view. Tschirch even went farther and considered the fungus hyphæ to represent nothing but preliminary stages in the differentiation of the cell plasma into bacteroids. Lecomte⁵³ considered that both the hyphæ and bacteroids were receptacles for the storage of albuminous material. Tschirch⁵⁴ calls the tissue in which the bacteroids are found "bacteroid tissue."

B. Frank⁵⁵ now unreservedly abandons his earlier views of the parasitic origin of the tubercles and holds it proved by Brunchorst that they represent peculiar organs of the Leguminosæ for the storage of proteid substance. He ascribes to the galls on *Alnus*, *Elæagnus*, etc., a similar role, while Brunchorst about this time in a preliminary paper⁵⁶ and later in a full presentation of his work on *Alnus* galls finds them to be due to a fungus.⁵⁷

Thus the question hung, as it were, in the balance at the close of what might be termed the middle period of investigations on this subject. But important contributions were soon to be published which would remove all doubt of the causal relationship of the soil organisms to the tubercles.

⁴⁷Studier öfver Leguminosernas rotknölar. Lund. 1874. Bot. Zeit. xxxv (1874). 381.

⁴⁸Ueber die biologische Bedeutung der Wurzelknöllchen bei den Papilionaceen. Jour. Landw. xxxiii (1885).

⁴⁹Ueber die Wurzelknöllchen der Leguminosen. Gesells. Naturw. Freunde in Berlin, and Bot. Centralb. xxxi (1887.)

⁵⁰Ueber die Knöllchen an den Leguminosen-Wurzeln. Bot. Centralb. xxix (1887). 53.

⁵¹Origine, structure et nature morphologique des tubercles radicaux des Légumineuses. Bull. d. Soc. Bot. France. xxxv (1888).

⁵²Zusammenstellung der neuern Arbeiten über die Wurzelknöllchen und deren als Bakterien angesprochene Inhaltskörperchen, Bot. Centralb. xxxi (1887). 308.

⁵³Bulletin d. l. Soc. Bot. d. France. 1888.

⁵⁴Beiträge zur Kenntniss der Wurzelknöllchen der Leguminosen. Ber. d. Deutsch. Bot. Gesells. v (1887). 58.

⁵⁵Sind die Wurzelanschwellungen der Erlen und Elæagnaceen Pilzgallen? Ber. d. Deutsch. Bot. Gesells. v (1887).

⁵⁶Ueber die Knöllchen an den Wurzeln von *Alnus* und den Elæagnaceen. Bot. Centralb. (1880). 354.

⁵⁷Ueber einige Wurzelanschwellungen, besonders diejenigen von *Alnus* und den Elæagnaceen. Unters. Bot. Inst. Tübingen. ii (1886). 151.

Recent period of investigation.

Ward⁵⁸ by a very careful series of inoculations with soil material proves conclusively that the tubercles are caused by some organism which is very abundant in the soil. Frank⁵⁹ had even earlier carried out similar experiments which pointed to like results. Ward traces the development of the fungus strands from infection in the root hairs, its growth down into the cortical parenchyma, and into the tissue of the tubercle, where it branches in all directions. He describes and figures the peculiar enlargements where the hyphæ pass through the cell walls, and also enlarged portions of the hyphæ within the cell lumen, characters which we have seen were noted by earlier observers.

On some of these enlarged portions he observed very small buds, or "gemmules," as he termed them, similar to those described by Frank. He considers these the points of origin of the bacteroids, in fact that the bacteroids, or gemmules, are budded off from the hyphæ. According to Ward these gemmules then increased by farther budding within the tubercle. Since there was some correspondence between this supposed development of the gemmules, or bacteroids, with the development of the sporids from the promycelia of some of the Ustilagineæ, and the successive buddings of the sporids in nutrient solutions, as shown by Brefeld,⁶⁰ Ward⁶¹ believed the fungus of the tubercles to be related to the Ustilagineæ, but through its peculiar symbiotism with its host, by which the "gemmules" could be preserved through the summer period and then during winter and spring set free by the decay of the tubercles, there was not the need of resting spores such as are found in the known Ustilagineæ, and consequently they had ceased to be produced. About the same time the cultural researches of Hellriegel,⁶² Hellriegel

⁵⁸On the tubercular swellings on the roots of *Vicia Faba*. Phil. Trans. Royal Society. CLXXVIII (1887). 139-562.

⁵⁹Bot. Zeitung, xxxvii. n. 24-25, 1879.

⁶⁰Botanische Untersuchungen. v. Die Brandpilze i. 1883.

⁶¹See also, On the tubercles on the roots of leguminous plants with special reference to the pea and bean. Proceed. Royal Society. XLVI. 1889.

⁶²Welche Stickstoffquellen stehen den Pflanzen zu Gebote? Tageblatt d. Naturf. Versamml. zu Berlin, 1886, p. 290.

and Wilfarth,⁶³ and Laws and Gilbert⁶⁴ confirmed the investigations of Ward, that the tubercles are due to the action of some organism within the soil.

Ward pointed out that the relation of the low organism to its host was that of a symbiosis, with mutual benefit. Lundström⁶⁵ entertains similar views, but left it undetermined whether the lower symbiont was a plasmodium or one of the bacteria.

In 1888 a very important contribution to the biology of the organism was presented by Beyerinck.⁶⁶ He undertook the cultivation of the organism in artificial media and claims to have carefully studied the development and morphology of several races or varieties, and in the tubercles he thought he could trace the development of the bacteroids from bacteria. The organism he considers one of the bacteria, and names it *Bacillus radicicola*. Besides the rod forms he describes a motile form of the organism which is very much smaller. He rejected the idea of the presence of fungus hyphae. These he considered to be the products of nuclear division. Not admitting a hypha as the means of infection he tried to explain the entrance of the organism and its passage from cell to cell by stating that there were invisible pores in the cell walls.

In 1888 Vuillemin⁶⁷ published his investigations, the results of which, while maintaining the relation of some symbiont in the tubercles, appear to be quite at variance with others concerning the nature of the organism. His studies of the development of the organism were directed to an examination of certain of the tubercles in the autumn. He observes the fungus threads and interprets the enlargements as sporangia. He claims to have observed the formation of zoospores in the sporangia. These he describes as pyriform, with a cilium at

⁶³Untersuchungen über die Stickstoffnahrung der Gramineen und Leguminosen. Beilageheft z. d. Zeitschr. f. d. Rübenzucker Ind. d. D. R. Berlin, Nov. 1888. Review in Bot. Centralb. xxxix (1889). 138.

⁶⁴On the present question of the sources of the nitrogen of vegetation, etc. Phil. Trans. Royal Society, CLXXX. B. 1-107. Etat actuel de la question des sources d'azote de la vegetation. Ann. Agr. xiv (1888).

⁶⁵Ueber symbiotische Bildungen bei den Pflanzen. Bot. Centralb. xxviii (1886).

⁶⁶Die Papilionaceenknoellchen, Bot. Zeit. 1888, p. 725-735, 741-750, 757-771, 780-790, 797-804.

⁶⁷Les tubercles radicaux des Légumineuses, Ann. d. Sci. Agr. Franç. et Etrang. 1888, p. 96.

the smaller end. They conjugate by fusion and form cysts. The non-septate character of the hyphæ taken in conjunction with the formation of zoospores, would ally the fungus with the *Chytridiaceæ* and Vuillemin considered it to be an undescribed species of the genus *Cladochytrium*, and called it *Cl. tuberculorum*. A comparison with Nowakowski's description and figures of *Cladochytrium tenue*⁶⁸ shows at least a striking resemblance in the form of the fungus as the hyphæ ramify through the tissues of *Acorus*, presenting here and there intercalary and terminal sporangia.

Vuillemin, with Ward,⁶⁹ Lundström⁷⁰ and Schindler,⁷¹ believes a symbiosis to exist between the fungus and the *Léguminosæ*. While Lundström classes the tubercles in the category of organs which are the products of mutual association, called by him "domatien,"⁷² Vuillemin⁷³ prefers the name suggested by Frank⁷⁴ and calls them "*Mycorhizes endotrophiques*," or "*endomycorhizes*." Ward⁷⁵ shows that their inclusion among the Mycorhizæ is as logical as that of Frank's inclusion⁷⁶ of Warlich's⁷⁷ orchid root fungus.

Frank later⁷⁸ distinctly places them, as well as the galls, on *Alnus*, *Elæagnus*, etc., among his Mycorhizæ.

⁶⁸Beitrag zur Kenntniss der Chytridiaceen, In Beiträge zur Biologie der Pflanzen, II (1876). 73-121.

⁶⁹Phil. Trans. Royal Society, CLXXVIII (1887). 539-562.

⁷⁰Ueber symbiotische Bildungen bei den Pflanzen, Bot. Centralb. XXVIII (1886).

⁷¹Zur Kenntniss der Wurzelknöllchen der Papilionaceen, Bot. Centralb. XVIII (1884). 86.

⁷²Ueber Mycodomatien in den Wurzeln der Papilionaceen, Bot. Centralb. XXXIII, 1888.

⁷³Remarques sur le memoire de Lundström. Jour. de Botanique, April, 1888.

⁷⁴Ueber die auf Wurzelsymbiose beruhende Ernährung gewisser Bäume durch unterirdische Pilze. Ber. d. deutsch. bot. Gesells. III (1885). 128-144. Neue Mittheilungen über die Mykorhiza der Bäume und der *Monotropa hypopitys*. Ber. d. deutsch. bot. Gesells. III (1885).

⁷⁵Some recent publications bearing on the question of the sources of nitrogen in plants. Annals of Botany, I. (1887-1888). 325-357.

⁷⁶Ueber neue Mykorhiza-Formen, Ber. d. deutsch. bot. Gesells. V (1887). 395-408.

⁷⁷Beitrag zur Kenntniss der Orchideenwurzelpilze. Bot. Zeitung. XLIV. (1886).

⁷⁸Ueber die auf Verdauung von Pilzen abzielende Symbiose der mit endotrophen Mykorhizen begabten Pflanzen, sowie der Leguminosen und Erlen. Ber. d. deutsch. bot. Gesells. IX (1891). 244-253.

Following close upon Vuillemin's contribution were several by A. Prazmowski, a preliminary paper in 1888,⁷⁹ in which he considers the tubercles to be due to a parasitic fungus related to Woronin's *Plasmodiophora brassicæ*.

A second paper appeared in 1889,⁸⁰ and the final results of his study of the etiology and development of the tubercles was published in 1890.⁸¹ Prazmowski now believes the tubercles to be due to one of the bacteria and he describes the organism obtained by himself in artificial cultures from the tubercles. By inoculation with the same he succeeded in developing the tubercles. According to him it is a bacterium. In artificial cultures it always develops in the form of simple short rods, which may for a time remain joined in short chains, but it never forms filaments. The rods may farther divide up into short cells, and it possesses a motile form. He denies the presence of fungus hyphæ, regarding them as gelatinous tubes which the organisms excrete to form a protective covering for the mass. The manner of infection through the root hairs agrees in all essential respects with that described by Ward (l. c.), only Prazmowski does not look upon the tube as having any genetic connection with the organism, but simply encloses them while the colony of bacteria directed by a common purpose move along together with the advancing tube down into the tissue of the root. In regard to this unity of purpose exhibited by the colony as described by Prazmowski, his organism would show a relationship to Thaxter's *Myxobacteriaceæ*.⁸²

Prazmowski was unable to demonstrate the membranous nature of the tube, while there seems to be pretty good evidence from the experience of Eriksson,⁸³ Ward,⁸⁴ Vuille-

⁷⁹Ueber Wurzelknöllchen der Leguminosen, Bot. Centralb. xxxvi (1886). 215-219, 248-255, 280-285.

⁸⁰O istocie i znaczeniu korzeniowych grochu. Berichte aus d. Sitz. d. k. k. Akad. d. Wissensch. in Krakau, June, 1889. Das Wesen und die biologische Bedeutung der Wurzelknöllchen der Erbse. Bot. Centralb. xxxix (1889). 356-362.

⁸¹Die Wurzelknöllchen der Erbse; Erster Theil, Die Ätiologie und Entwicklungsgeschichte der Knöllchen. Landw. Versuchs-Stationen. xxxvii (1890). 160-236.

⁸²On the Myxobacteriaceæ, a new order of Schizomycetes. Bot. Gazette, xvii (1892). 12.

⁸³Studier öfver Leguminosernas rotknölar. Lund. 1874. Bot. Zeit. xxxv. (1874). 381.

⁸⁴Phil. Trans. Royal Society. CLXXVIII. (1887). 539-562. Proceed. Royal Soc. XLVI (1889). 431-443.

min,⁸⁵ Pichi,⁸⁶ A. Koch,⁸⁷ and Laurent⁸⁸ that a membrane does exist.

Short communications by Delpino⁸⁹ and Mattei⁹⁰ about this time support the view that the tubercles are caused by bacteria.

In 1890⁹¹ Frank appears with still another contribution. While he does not wholly return to his earlier convictions he retraces his steps far enough to once more champion the causal relation of some soil organism. This time he believes the organism to be micrococoid and names it *Rhizobium leguminosarum*. He still recognizes the two form elements in the tubercles, the hypha-like strands and the bacteroids. The former he believes to be a homogeneous mixture of the cell protoplasm of the two symbionts, in which nothing is visible indicative of the nature of the *Rhizobium* or the protoplasm of the leguminous plant. This mixture he terms *mycoplasm*. Upon treatment with certain reagents the portion of the mycoplasm belonging to the host plant dissolves and sets the micrococci free. Frank also obtained his *Rhizobium* in artificial culture and produced the tubercles by inoculation with the same. The bacteroids he regarded as fragments of the mycoplasm, while in a more recent communication⁹² he says they are hypertrophied forms of the *Rhizobium*.

In the same year Laurent published a preliminary paper⁹³ on this subject, followed in 1891⁹⁴ by a more complete exposition of his investigations. He also obtained the organism in artificial cultures and recognizes the genetic connection of the bacteroids and fungus strands, believing with Ward and the earlier tenets of Frank that the bacteroids are budded off

⁸⁵ Ann. d. Sci. Agr. Franç. et étrang. 1888. p. 96.

⁸⁶ Alcune osservazioni sui tubercoli radicali delle Leguminose. Atti della Società Toscani di science naturali. 1888.

⁸⁷ Zur Kenntniss der Fäden in den Wurzelknöllchen der Leguminosen.

⁸⁸ Ann. d. l'Institut Pasteur. v. (1891). 105-139.

⁸⁹ Osservazioni sopra batteriocecidii e la sorgente l'azote in una piante Galega officinalis. Malpig. II. fasc. 9-10. (1889). Cited by Prazmowski.

⁹⁰ Ancora sull' origine della Vicia faba. Bologna. 1887. Cited by Vuillemin.

⁹¹ Ueber die Pilzsymbiose der Leguminosen. Berlin, 1890.

⁹² Berichte d. deutsch. bot. Gesellsch. IX. (1891). 244-253.

⁹³ Sur le microbe des nodosités des Légumineuses. Compt. Rend. d. Sc. Paris. 1890. pp. 754-756.

⁹⁴ Recherches sur les nodosités radicales. Ann. d. l'Inst. Pasteur. v. (1891) 105-139.

from the hyphæ. He treats of the organism as a yeast form, and while in his artificial cultures a large number of the individuals were rod-like, some presented an irregularly lobed form. These forms together with the development of the bacteroids from the hyphæ he judges separates the organism from the true *Schizomycetææ*. He accepts Frank's name *Rhizobium leguminosarum*, but believing it to be related to Metschnikoff's *Pasteuria ramosa*,⁹⁵ he locates it in the *Pasteuriaceæ*.

During the last year an article on this subject has appeared from an American writer.⁹⁶ The original part of Schneider's paper deals with the morphology of the bacteroids of several leguminous species, the variations in form of the bacteroids alone seeming in the estimation of the author sufficient ground for the characterization of species, and several species and varieties are named. Schneider accepts Frank's generic name *Rhizobium*, but rejects his theory of a mycoplasm. He denies the genetic connection of the bacteroids with the hyphæ, and definitely rejects the idea of any causal relation of the fungus hyphæ to the tubercles. He observes fungus threads in the tubercles, but cannot differentiate them from fungus threads which he finds in other parts of the roots where there are no tubercles.

[To be concluded.]

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BRIEFER ARTICLES.

Two new plants from Washington.—*Allium Hendersoni*.—About a foot high: bulb white, ovate, 6–7 lines in diameter, finely but indistinctly reticulated: leaves 2, linear-oblong, attenuate, thickish, 4–6 inches long, as many lines broad: scape rather stout, much exceeding the leaves: umbels many-flowered, globose: spathe 3-parted; bracts ovate, acuminate, 6–8 lines in length: pedicels 8–10 lines long: segments of the perianth rhombic-ovate, acuminate, 3–4 lines long, purple, with light midrib: stamens exserted: ovary 6-crested: seeds dull black, compressed, obovate, $1\frac{1}{2}$ lines in length.—Collected near Pull-

⁹⁵*Pasteuria ramosa*, un représentant des bactéries à division longitudinale. *Ann. d. l'Inst. Pasteur*. II. (1888.) 165–170.

⁹⁶Observations on some American *Rhizobia*. *Bull. Torrey Bot. Club* XIX. July, 1892.